Effect of a Low-Intensity Pulsed Ultrasound Device, SX-1001, on Clinical Symptoms in Buerger Disease With Limb Ischemia

A Multicenter, Double-Blind, Parallel Randomized Clinical Trial

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SUMMARY

Buerger disease is a rare disease of unknown etiology and cannot be treated by bypass surgery or percutaneous re-endothelialization. Although the need for effective limb ischemia prevention strategies is increasingly being recognized, effective preventative strategies are insufficient. The aim of this study using a new pulsed ultrasound device, SX-1001, is to determine whether treatment using SX-1001 can mitigate rest pain and improve blood supply to ischemic legs in patients with Buerger disease. This study is a multicenter, double-blinded, parallel randomized clinical trial testing the efficacy and safety of SX-1001. Treatment using SX-1001 is expected to result in reduction of the visual analog scale score for pain in Buerger disease patients who have Fontaine stage III. A total of 44 patients from 20 hospitals in Japan will be enrolled. The primary endpoint of the trial is a change in rest pain intensity on the visual analog scale score from baseline to 24 weeks. This trial will be the first to show the safety and efficacy of low-intensity pulsed ultrasound using SX-1001 for clinical symptoms in patients with Buerger disease. Low-intensity pulsed ultrasound may be a new therapy for limb ischemia. Ethical approval has been obtained from each of the participating institutes. Study findings will be disseminated through peer-reviewed journals and at scientific conferences.

This study is registered at UMIN Clinical Trial Registry (UMIN000014757). (Int Heart J 2015; 56: 632-638)

Key words: Peripheral arterial disease, Perfusion parameters, Walking performance, Rest pain intensity, Visual analog scale score

Recently, the concept of angiogenesis has provided a new option for critical limb ischemia (CLI) treatment. Mechanisms of vascular development and differentiation have been studied, and it has been clarified that angiogenesis is induced by differentiation and proliferation of endothelial progenitor cells (EPCs) or by EPCs mobilized from bone marrow and peripheral blood as well as by proliferation and migration of EPCs from existing blood vessels.¹⁻⁵ Several investigators have shown the efficacy of gene therapies using vascular growth factors or cell therapies for angiogenesis in patients with CLI.⁶⁻¹³ Unfortunately, both gene and cell therapies are not always satisfactory in patients with peripheral arterial disease (PAD). A more effective and simple way for inducing

angiogenesis is needed.

Low-intensity pulsed ultrasound irradiation has been widely used to promote the healing of bone fractures in humans.¹⁴⁻¹⁶ It is postulated that the initial step of bone fracture healing by low-intensity pulsed ultrasound is angiogenesis, and subsequent neovascularization leads to the migration of osteoblasts to the bone fracture site. Indeed, some studies have shown that low-intensity pulsed ultrasound induces angiogenesis in experimental hind-limb ischemic models.¹⁷⁻¹⁹ However, there is no information on the effects of pulsed ultrasound on angiogenesis and clinical symptoms in humans. We hypothesized that low-intensity pulsed ultrasound irradiation has a beneficial effect on limb ischemia and improves clinical symp-

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toms by inducing angiogenesis in patients with limb ischemia. Thus, we developed a novel ultrasound device for treatment of limb ischemia. The efficacy of the device for improving clinical symptoms has not yet been tested.

Buerger disease is a rare disease of unknown etiology and cannot be treated by bypass surgery or percutaneous re-endothelialization. Therefore, Buerger disease is a good target for therapeutic angiogenesis. Recently, we have shown that cell therapy with bone-marrow mononuclear cell implantation is safe and effective in patients with CLI, especially in patients with Buerger disease.²⁰⁾ In this trial, we are enrolling Buerger disease patients who have Fontaine stage III.

This trial is a verification study of the effect and safety of pulsed ultrasound for cases of limb ischemia that do not respond to conventional oral administration or surgical treatment or for which conventional treatments are not suitable.

METHODS

Design: This study is a double-blinded, dummy-controlled parallel-group ultrasound therapy trial using a newly developed device, SX-1001, in Buerger disease patients who have Fontaine stage III. For enrolment, patients must have limb ischemia with ischemic lesions. The eligibility of a patient will

Figure 1. Design schematic of time schedule of the trial.

be confirmed by the independent Case Assessment Committee consisting of specialists of vascular surgery, cardiology, radiology, and stroke before starting ultrasound treatment. Standard therapy recommended by Trans Atlantic Inter-Societal Consensus II (TACS II) will be maintained during the entire study period without changing the doses of concomitant medications or physical therapy program. The trial design and schedule are presented in Figure 1 and Table I, respectively. All patients are reviewed prior to study and at 4, 8, 12, 16, 20, and 24 weeks during the treatment phase for assessment of both efficacy and safety.

Study population: Buerger disease will be diagnosed by the previously described criteria,²¹⁾ including results of physical examinations, clinical symptoms, and angiographic findings: smoking history, onset before the age of 50 years, infrapopliteal arterial occlusive disease, either upper limb involvement or phlebitis migrans, and absence of atherosclerotic risk factors other than smoking. To rule out other vasculitis and hypercoagulable states, rheumatoid factor, lupus anticoagulants, and serologic investigations will be evaluated. The diagnosis of limb ischemia will be confirmed by angiography. Limb ischemia will be classified according to the guidelines of TASC II.²²⁾ The target population is patients with Buerger disease in the lower extremity region who have Fontaine classification III. Patients have below the knee disease based on angiographic findings. Patient age will be limited to ≥ 20 years and < 65 years. Patients must quit smoking for at least 3 months and be negative for gas chromatography (GC)/mass spectrometry (MS) screening with a plasma cotinine level over 10 ng/mL during enrolment. A total of 44 patients will be enrolled in this study and they must maintain a constant lifestyle throughout the study. Inclusion criteria are shown in Table II, and exclusion criteria are shown in Table III. The study protocol has been approved by the relevant institutional review boards. Written informed consent for participation in the study will be obtained from all subjects.

Randomization and masking: Eligible patients with Fontaine III are randomly assigned to receive treatment with either active ultrasound equipment (SX-1001, Nippon Sigmax Co., Ltd,

Table I. Trial Schedule

Parameter	Screening until day 0	Treatment period (weeks)					
		4	8	12	16	20	24
Informed consent	○						
Enrolment	○						
Clinical characteristics	○						
Smoking context	○	○	○	○	○	○	○
Smoke-out screening	○	○	○	○			
Systemic hemodynamics	○	○	○	○	○	○	○
Hematologic test	○	○	○	○			
Biochemical test	○	○	○	○			
Urinary test	○	○	○	○			
Pregnancy test	○						
Subjective and objective symptoms	○	○	○	○	○	○	○
Visual analog scale	○	○	○	○	○	○	○
Transcutaneous oxygen pressure	○			○			
Skin perfusion pressure	○			○			
Ankle brachial pressure index	○			○			
Toe brachial pressure index	○			○			
Walking distance	○			○			
Adverse event		○	○	○	○	○	○

Table II. Inclusion Criteria

Variable
Aged 20 years or over and less than 65 years
Meeting the diagnosis criteria of the specified and intractable vasculitic disease study group of the Japanese Ministry of Health and Welfare
Signed informed consent
Limb ischemia of Fontaine stage III
Below the knee disease of angiographic findings
Non-smoking during the study period confirmed by plasma cotinine level
Non-improvement of symptoms of Buerger disease with conventional therapy recommended by Trans-Atlantic Inter-Society Consensus (TASC) II
Outpatient visits during the 24 weeks of treatment

Table III. Exclusion Criteria

Variable
Planned major amputation during the study period
Possibility of successful by-pass surgery or catheter treatment on the index leg
Applicability of by-pass surgery or catheter treatment on the index leg
Upper knee disease based on angiographic findings
Active malignant disease, severe malfunction of the liver or kidneys and the forefoot evidenced by imaging (radiography)
Not expected to survive more than 12 months, in the opinion of the principal investigator of the study site
A history of malignant disease or previous or present history of cardiac infarction or stroke within 3 months before recruitment
Cardiovascular diseases of New York Heart Association class III or IV or active angina
Current or planned pregnancy during the study period
Previous treatment in another investigational clinical trial within 6 months before recruitment
Heavy second-hand tobacco smoke or patients excluded based on the opinion of the principal investigator of the study site

Tokyo) or dummy equipment (ratio 1:1). The random code list is electrically generated by an independent trial statistician where the clinical center is assigned to a block. Randomization is managed by a third-party enrolment center.

Procedures: Subjects will be given detailed instructions and a manual for operation of the equipment and instructed to use the equipment on the index leg daily at home 20 minutes every day for 24 weeks. Operation records including starting and completion times will be automatically recorded by the logger in the equipment. Subjects will be advised to visit the outpatient clinic once every 4 weeks. Ultrasound irradiation will be applied over the skin of the ischemic area of the calf region for 20 minutes per day during the 24-week period. The gap between the transducer and skin will be filled with ultrasonic gel. A transducer (Nippon Sigmax Co., Ltd, Tokyo) generates a pulsed 2.0-MHz signal with a 200- μ second pulse burst and 1-kHz repetition rate at the intensity of 30 mW/cm² and a pulse wave with a duty cycle of 20%. In this trial, 8 transducers will be attached to the skin over the gastrocnemius of each ischemic leg (Figure 2). If the disease has affected both legs and both are unsuitable for re-vascularization, the leg with the lower ankle brachial pressure index (ABI) will be identified as the leg

to be treated (index leg). The adverse effects and worsening symptoms for the other leg will be recorded in the case report form.

Endpoints: Primary and secondary endpoints are shown in Table IV. The primary endpoint of the trial is the change in rest pain intensity on a visual analog scale (VAS) from baseline to 24 weeks. The secondary endpoints are the change in rest pain intensity on the VAS from baseline to 12 weeks, changes in transcutaneous oxygen pressure (TcPO₂), skin perfusion pressure (SPP), ABI, toe brachial pressure index (TBI), and walking distance from baseline to 12 and 24 weeks, and changes in symptoms related to Buerger disease in the study period of 24 weeks.

Measurements:

Primary endpoint Rest pain intensity is assessed by a self-administered VAS from baseline to 24 weeks as previously described.²³⁻²⁵ Briefly, observers place the VAS line at a point that represents the pain intensity of respondents. The respondents complete the VAS line presentation by themselves within 1 minute. The VAS score is a horizontal line of 10 cm in length, with 0 cm corresponding to no pain and 10 cm corresponding to most severe pain. The observers are blind to the form of ex-

Figure 2. **A:** Appearance of the low-intensity pulsed ultrasound device. **B:** Application of transducers for LIPUS irradiation in the gastrocnemius of the ischemic leg.

Table IV. Endpoints

Variable	
Primary endpoint	· Change in rest pain intensity on a visual analog scale (VAS) from baseline to 24 weeks
Main secondary endpoints	· Change in rest pain intensity on a VAS from baseline to 12 weeks · Changes in transcutaneous oxygen pressure (TcPO ₂), skin perfusion pressure (SPP), ankle brachial pressure index (ABI), toe brachial pressure index (TBI) and walking distance from baseline to 12 and 24 weeks · Changes in symptoms related to Buerger disease in the study period of 24 weeks

amination.

Secondary endpoints

1) Rest pain: Rest pain intensity is assessed by a self-administered VAS from baseline to 12 weeks.

2) Perfusion parameters: All of the studies will be performed in the morning, after overnight fasting, in a quiet, dark and air-conditioned room (constant temperature of 22–25°C). The subjects will remain in a supine position throughout the study. Perfusion parameters are measured after a 15-minute rest period. TcPO₂ will be measured by an electrochemical transducer (TCM 400, Radiometer K.K., Tokyo) as previously described.^{26,27} Briefly, skin at the measured site will be shaved and cleaned with alcohol to ensure optimal TcPO₂ electrode contact for skin oxygen diffusion. The TcPO₂ electrodes stabilized at 43°C will then be placed on the skin at the measured site. TcPO₂ values will be recorded after a 20-minute stabilization period for the electrodes. SPP will be measured by a SensiLase PAD3000 flowmeter (Kaneka Medix Co., Osaka, Japan) as previously described.²⁸ Briefly, a laser Doppler probe positioned within the blood pressure cuff will be wrapped around the subject's foot. ABI and TBI will be measured with a new device (Form PWV/ABI; Omron Colin, Co., Tokyo) as previously described.^{29,30} Briefly, the subjects will be kept in the supine position for at least 5 minutes and cuffs will be wrapped around both brachia and ankles. An oscillometric method is used to measure systolic blood pressure in the bilateral brachial and posterior tibial arteries. The ABI value is calculated by dividing the highest pressure in the posterior tibial

arteries on the right and left sides by the highest brachial pressure on either side. To measure toe pressure, appropriately sized cuffs will be placed at the base of the toe. The TBI value is calculated by dividing the highest pressure in the toe on the right and left sides by the highest brachial pressure on either side. All procedures will be performed in accordance with the manufacturer's protocol. The observers will be blind to the form of examination.

3) Walking performance: Maximal walking distance/time or pain-free walking distance/time or both will be measured to evaluate walking performance by a treadmill test under the conditions of 2.4 km/h and 12% degrees in incline of the treadmill device, and the maximum walking distance will be measured up to 400 m during a period of 10 minutes.

Others

1) Clinical characteristics: Before starting exposure, the following will be checked and recorded: primary doctor, medical record number, patient's initial, sex, birth date, height, body weight, diagnosed disease, risk factors for coronary artery disease, smoking history, family history, treatment upon enrolment, in-house or out-patient, and concomitant diseases.

2) Smoking-out screening test: Smoking-out is confirmed by a negative result for GC/MS screening with a plasma cotinine level over 10 ng/mL during enrolment.

3) Systemic haemodynamics: Systolic pressure, diastolic pressure, and heart rate will be measured by the standard sphygmomanometric method in each treatment period.

4) Hematologic and biochemical tests : Baseline fasting levels

of hematocrit, hemoglobin, red blood cells, white blood cells, white blood cell fraction, and platelets and fasting serum concentrations of aspartate aminotransferase, alanine aminotransferase, bilirubin, alkaline phosphatase, gamma-guanosine triphosphate, creatine kinase, serum protein, serum albumin, C-reactive protein, total cholesterol, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, glucose, and HbA1c will be measured.

5) Urinary test: Baseline fasting urinary concentrations of urinary protein, albumin, and creatinine will be measured.

6) Pregnancy test: Before starting exposure, a pregnancy test using measurement of urinary excretion of human chorionic gonadotropin will be performed in pre-menopausal women.

7) Adverse effects : Abnormal laboratory data will be included in adverse effects. The primary doctor will give necessary treatment and fully evaluate the follow-up process. The primary doctor will record adverse effect details, date of onset and/or disappearance, degree, treatment, outcome, severity, and causal relationship to the low-intensity ultrasound exposure in the medical record. Except for unrelated adverse effects, the patient will be followed up as long as possible until recovery. Safety assessments will include all adverse and serious adverse events, subjective symptoms, and blood tests. A history of cancer or an active tumor will be an exclusion criterion (Table III).

Equipment: Figure 2A shows the newly developed pulsed ultrasound device, SX-1001.

Specifications

- 1) Size: 249 mm (W) × 298 mm (D) × 142 mm (H)
- 2) Weight: 1.9 kg
- 3) Power: AC100 V ± 10%
- 4) Apparent power: 35 VA or less
- 5) Protection Class for electroconvulsion: Class II
- 6) Protection Type for electroconvulsion: Type BF
- 7) Ultrasound specifications:
 - Channels: 8 channels
 - Acoustic Frequency: 2 MHz ± 10%
 - Intensity (Isata): 30 mW/cm² ± 10%
 - Pulse Repetition Frequency (PRF): 1 msec ± 5% (1 kHz ± 5%)
 - Duty: 20 ± 5%

Dummy equipment Dummy equipment is the same size and weight as that of the SX-1001. It operates in the same way as the SX-1001. The dummy shows the same display as that of SX-1001 during operation and cannot be distinguished from the active equipment by the study subjects or the investigator.

Compliance After treatment of 20 minutes, both devices stop operating automatically. A daily record of operation starting and completion times will be stored in the logger in the equipment during the entire study period for assessment of treatment compliance.

Application of transducers Figure 2B shows the application of transducers for low-intensity pulsed ultrasound irradiation in the gastrocnemius of the ischemic leg.

Data analysis:

Sample size estimation This study hypothesized that the SX-1001 would reduce the rest pain score as compared to the dummy instrument during the 24 weeks of the treatment period. The difference in the decrease of the pain score during the 24 weeks between the two groups was assumed to be 1.0 with a standard deviation of 1.1 based on a preliminary study. To detect such a difference statistically, 22 patients per group will

be required to achieve 80% power assuming an attrition of 10%.

Statistical analysis plan Mean difference in the absolute change of the rest pain score during the 24 weeks will be compared between the two groups by the analysis of covariance with a baseline score as a covariate. A missing value of the rest pain score at 24 weeks will be imputed according to the principle of the last observation carried forward. A mixed-effects model will be also applied to the repeated measured data as a sensitivity analysis. Similar analyses will be applied to the secondary endpoints of TcPO₂, SPP, TBI, and walking distance. Foot ulcer elimination rates will be compared between the two groups using Fisher's exact test. Statistical significance will be assessed at a two-sided 5% level.

DISCUSSION

This trial will be the first study showing the safety and efficacy of low-intensity pulsed ultrasound for clinical symptoms in patients with Buerger disease using a randomized, double-blinded, and placebo-controlled design. Low-intensity pulsed ultrasound may be a new therapy for limb ischemia.

In a clinical setting, low-intensity pulsed ultrasound irradiation has been widely used to promote the healing of bone fractures.¹⁴⁻¹⁶⁾ Angiogenesis should be the initial step of bone fracture healing by low-intensity pulsed ultrasound, and subsequent neovascularization leads to the migration of osteoblasts to the bone fracture site. Although there is no information on the effects of low-intensity pulsed ultrasound on angiogenesis in humans, a few studies have shown that low-intensity pulsed ultrasound induces angiogenesis in experimental hind-limb ischemic models.¹⁷⁻¹⁹⁾ It is expected that low-intensity pulsed ultrasound irradiation will improve clinical symptoms by inducing angiogenesis in patients with Buerger disease.

Ultrasound induces an increase in shear stress on the surface of endothelial cells.³¹⁾ It is well known that acute or chronic increases in shear stress patently stimulate the release of nitric oxide (NO) in isolated vessels and cultured cells.^{32,33)} Therefore, ultrasound also may act as a mechanical stimulator of NO release. Indeed, Suchkova, *et al*¹⁸⁾ have shown that vasodilatory effects of low-intensity pulsed ultrasound are completely abolished by an NO synthase inhibitor in a rabbit hind-limb ischemic model. This beneficial effect of low-intensity pulsed ultrasound irradiation may also be expected in patients with PAD.

It is thought that low-intensity pulsed ultrasound, *in vitro* and *in vivo*, induces angiogenesis through increases in angiogenic growth factors and angiogenic cytokines such as interleukin-8, basic fibroblast growth factor, and vascular endothelial growth factor.^{34,35)} Angiogenic transcription factors, angiogenic cytokines and their receptors would contribute to ultrasound-induced angiogenesis. The therapeutic range of ultrasound stimulates both NO and prostaglandin E2 synthesis in human osteoblasts.³⁶⁾ Exposure of endothelial cells to therapeutic ultrasound increases NO synthase activity and NO production, which could lead experimentally or therapeutically to the induction of vasodilatation.³⁷⁾ Ultrasound irradiation of 1.6-2.0 W/cm² in intensity for 6 days enhanced NO and Ca²⁺ release from endothelial cells but did not promote endothelial cell growth.³⁸⁾ In addition, ultrasound irradiation altered cellu-

lar morphology and orientation and increased extracellular matrix secretion from endothelial cells.¹⁶⁾ It is expected that a benign circle among angiogenesis, improvement of endothelial function, and increase in NO promotes more effective angiogenesis during low-intensity pulsed ultrasound irradiation, leading to improvement of clinical symptoms.

Study limitations: There are a number of limitations concerning assessment of pain using a VAS. In a large number of trials for assessment of chronic pain, such as rheumatoid arthritis and osteoarthritis, VAS is used as the primary endpoint.^{39,40)} A good agreement between VAS score and patient-reported pain reduction has been shown in patients with cancer-related pain.⁴¹⁾ However, there is no internationally agreed upon definition of pain scale in patients with PAD. In our trial, we selected VAS as the primary endpoint since the subjects are patients with Buerger disease who have Fontaine III. We believe that a visual pain scale is a suitable endpoint in this trial. It has been reported that a clinical improvement in pain using the Global Rating Scale relates to a 30% reduction in pain.⁴²⁾ Rosier, *et al*⁴³⁾ have shown that repeated VAS measurements have a variability of approximately 20% when evaluating reproducibility of pain measurement using a VAS. It is thought that reduction in VAS of 30% means a significant decrease in pain status. In a preliminary study, we confirmed that low-intensity pulsed ultrasound decreased VAS from 6.4 ± 0.8 (SD) to 4.1 ± 0.6 (SD) ($P < 0.01$) in 12 patients with Buerger disease who have Fontaine III and IV. However, unfortunately, since there is no golden standard for validity of pain criteria at present, we do not know what a change of 1 in VAS score actually means, especially in patients with PAD. Further studies are needed to develop and validate a standardized VAS measurement.

In conclusion, low-intensity pulsed ultrasound may be a new strategy for improving ischemic limb symptoms. If low-intensity pulsed ultrasound is shown in this trial to be effective for improving clinical symptoms in patients with Buerger disease who have limb ischemia, further studies will be needed to confirm the efficacy of low-intensity pulsed ultrasound for improving clinical symptoms in atherosclerotic patients with PAD and in patients with mild Buerger disease and severe Buerger disease with ulcers and gangrene. In addition, it is expected that low-intensity pulsed ultrasound irradiation to the heart, kidney and liver will be useful for improvement of tissue injury and clinical symptoms in patients with coronary heart disease, kidney disease, and liver disease. The door will be open to expand ultrasound beyond the field of diagnostic imaging.

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